Residue Method for the Determination of NOA449280 and Metabolite SYN503780 in Water

Data Requirement: EPA Guideline: 850.6100
OECD Data Point: IIA 4.5

Reports:

Analytical Method: MRID 47841954. Crook, S. 2008. ECM: NOA449280: Residue Method for the Determination of NOA449280 and Metabolite SYN503780 in water. Final Determination by LC-MS/MS. Report No. GRM030.01A. Unpublished study prepared by Syngenta Ltd., Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, UK. Submitted by Syngenta Crop Protection, LLC, Greensboro, NC.

Independent Laboratory Validation: MRID 47841955. Eversfield, S. and Morriss, A. 2008. NOA449280 – Validation of a Method (Draft GRM030.01A) for the Determination of NOA449280 and SYN503780 in Drinking, Surface and Ground Water. Report No. CEMR-3547. Unpublished study prepared by CEM Analytical Service (CEMAS), Glendale Park, Fernbank Road, North Ascot, Berkshire SL5 8JB. Submitted by Syngenta Crop Protection, LLC, Greensboro, NC.

Independent Laboratory Validation: MRID 47842122. Amic S. 2012. NOA449280: Independent Laboratory Validation of Analytical Method GRM030.01A for the Determination of NOA449280 and SYN503780 residues in Water. Report No. S11-03839. Unpublished study prepared by Eurofins|ADME BIOANALYSES 75 Chemin de Sommières 30310 Vergèze, France. Submitted by Syngenta Crop Protection, LLC, Greensboro, NC.

Compliance Statements: The reports stated that these studies were conducted in compliance with GLP practices.

Classification: This environmental chemistry method is classified as **Fully Reliable** (EPA classification: Acceptable) with respect to determination of NOAA449280 and metabolite SYN503780 in water. However, under EPA data requirements, a data gap remains. While the initial method validation demonstrates that the analytical chemistry method for the analysis the parent and one metabolite in water is valid, Independent Laboratory Validation (ILV) data were submitted only for groundwater; the method was not independently validated for drinking water or surface water. The other submitted ILV study was not an independently conducted method validation, but appeared to report the exact same data as in the initial method validation. Additionally, these studies do not address the other major metabolites of bicyclopyrone. Additional studies (MRIDs 47842016, 47841956) were submitted that do address the major metabolites of bicyclopyrone, but they also did not include sufficient ILV data.

PC Code: 018986

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EXECUTIVE SUMMARY

This analytical method is designed for the quantitative determination of NOA449280 (parent bicyclopyrone; CAS# 352010-65-5) and its metabolite SYN503780 (CAS# 380355-55-5) in water. The analytical chemistry method involves acidifying a well mixed water subsample and adding it to a Strata-X SPE cartridge, discarding the eluate. The analytes are sorbed to the column and subsequently quantitatively eluted with acetonitrile. They are appropriately diluted for analysis by LC-MS/MS. Review of this method indicated that the initial method validation is acceptable. However, one of the supporting Independent Laboratory Validation (ILV) studies (MRID 47841955) appears to consist of the exact same data reported for the initial method validation. The second ILV study (MRID 4781955) uses only a single sample type (groundwater) to assess the analytical methodology, and does not validate the method for drinking water or surface water, as the initial method validation does.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Lab Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validations						
NOA449280 SYN503780	47841954	47841955 47821222	None	Water	09/04/2007	Syngenta	LC/MS-MS	0.01 µg/L

I. PRINCIPLE OF THE METHOD

This method starts with a 10 ml sample of thoroughly mixed water. The 10 ml aliquot is then placed in a centrifuge tube and 200 µl of formic acid is added to each sample (pH must be <2, checked with suitable indicator paper). Use one Strata-X SPE cartridge per sample. Place in a vacuum manifold. Add 2 ml methanol percolate or draw through column to level of top frit discarding the eluate. Add samples to column allowing flow thru rate of 1-2 ml per minute to level of top frit. NOA 449280 and SYN 503780 are retained on column. At no time should column be allowed to dry. Rinse tube with ultra pure water plus 2ml of 2% formic acid and add to column. Elute as before. Elution of NOA 449280 and SYN 503780 is accomplished adding 3 ml of methanol that is percolated or drawn through the column under low vacuum collecting the column eluate. Eluate is evaporated to dryness with clean air. Immediately upon dryness acetonitrile (200 µl) is added and the sample ultrasonicated. Ultra-pure water (0.8 ml) is added and the sample again ultrasonicated until completely dissolves and mixed. Analysis is by LC-MS/MS.

II. RECOVERY FINDINGS:

Table 2. Initial Validation Method Recoveries for Analytes in Drinking Water

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
NOA449280 Primary transition	0.01	5	91-96	94	n/a	2.1
	0.1	5	89-93	91	n/a	1.7
SYN503780 Primary transition	0.01	5	82-99	89	n/a	7.1
	0.1	5	86-91	89	n/a	2.3
NOA449280 Confirmatory transition	0.01	5	86-107	92	n/a	9.8
	0.1	5	93-99	97	n/a	2.7
SYN503780 Confirmatory transition	0.01	5	81-96	88	n/a	8.6
	0.1	5	90-97	95	n/a	3.2

Table 3. Initial Validation Method Recoveries for Analytes in Surface Water

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
NOA449280 Primary transition	0.01	5	78-86	83	n/a	3.7
	0.1	5	89-93	91	n/a	1.7
SYN503780 Primary transition	0.01	5	85-96	92	n/a	4.8
	0.1	5	92-100	97	n/a	3.0
NOA449280 Confirmatory transition	0.01	5	68-116	87	n/a	21.7
	0.1	5	76-80	77	n/a	2.1
SYN503780 Confirmatory transition	0.01	5	87-99	95	n/a	4.9
	0.1	5	95-103	99	n/a	3.3

Table 4. Initial Validation Method Recoveries for Analytes in Ground Water

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
NOA449280 Primary transition	0.01	5	81-88	84	n/a	3.0
	0.1	5	88-94	91	n/a	2.8
SYN503780 Primary transition	0.01	5	90-100	93	n/a	4.4
	0.1	5	93-99	96	n/a	2.3
NOA449280 Confirmatory transition	0.01	5	84-101	93	n/a	6.8
	0.1	5	86-99	93	n/a	6.1
SYN503780 Confirmatory transition	0.01	5	89-105	97	n/a	6.3
	0.1	5	94-101	97	n/a	4.7

Table 5. Independent Validation Method Recoveries for Analytes in Ground Water

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
NOA449280 Primary transition	0.01	5	79-86	82	n/a	3
	0.1	5	82-84	83	n/a	1
SYN503780 Primary transition	0.01	5	79-86	82	n/a	3
	0.1	5	84-87	85	n/a	1
NOA449280 Confirmatory transition	0.01	5	75-82	79	n/a	3
	0.1	5	80-82	82	n/a	1
SYN503780 Confirmatory transition	0.01	5	70-81	74	n/a	6
	0.1	5	82-86	83	n/a	7

III. METHOD CHARACTERISTICS:

Table 6. Method Characteristics

	NOA449280	SYN503780
Limit of Quantitation (LOQ)*	0.01 µg L^{-1}	0.01 µg L^{-1}
Limit of Detection (LOD) primary ion	0.002 µg L^{-1}	0.02 µg L^{-1}
Limit of Detection (LOD) confirmatory ion	0.005 µg L^{-1}	0.005 µg L^{-1}
Linearity (calibration curve r^2 and concentration range)	$r^2 = 0.99$ 0.05 ng/mL– 10 ngm/L	$r^2 = 0.99$ 0.05 ng/mL– 10 ngm/L
Repeatable	Yes	Yes
Reproducible	Yes	Yes
Specific	Yes	Yes

* The limit of quantitation of the method is defined as the lowest analyte concentration in a sample at which the methodology has been validated and a mean recovery of 70-110% with a relative standard deviation of ≤ 20% has been obtained.

IV. METHOD DEFICIENCIES AND REVIEWER'S COMMENTS:

The study presented in MRID 47841955 as an Independent Laboratory Validation (ILV) appears to be the exact same data reported for the initial method validation in MRID 47841954. As such, it does not meet the criteria to be an independent validation of the analytical method.

The original method was run with three different types of water samples, drinking ground and surface water. The other submitted ILV study, MRID 47842122 has only a single sample type (groundwater) run by this methodology. In the initial method validation study it is stated that the method produced acceptable results for ground water hence it may "reasonably assumed" that the method will work in other matrices. However, these different matrices present differing analytical challenges, and further testing, especially in surface water, would give more confidence in the methodology.

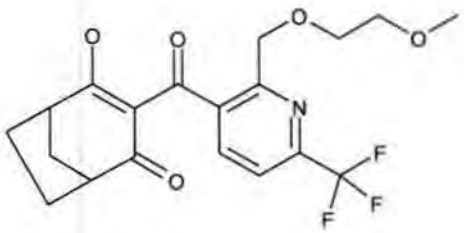
The study authors indicated that the study was conducted according to EPA OPPTS Guidelines 860.1340 and 850.7100; and EC Guidelines SANCO/825/00 rev 7 and SANCO/3029/99 rev 4. The studies actually fall under EPA Guideline 850.6100 at the time of this review.

This method is designed for the quantitative determination of residues of bicyclopyrone (NOA449280) and its metabolite S YN503780 in water. The initial validation of the environmental chemistry method meets the criteria for a scientifically valid method for the parent and the single metabolite studied. However, the ILVs submitted do not meet guideline criteria, as the one ILV was not an independently performed validation of the methods based on the fact that the data submitted were identical to the data for the initial method validation. The second ILV was conducted only on one type of water (groundwater) and did not independently

validate the method in either drinking water or surface water.

Attachment 1: Chemical Names and Structures:

IUPAC Name:	**4-Hydroxy-3-[2-(2-methoxy-ethoxymethyl)-6-trifluoromethyl-pyridine-3-carbonyl]-bicyclo[3.2.1]oct-3-en-2-one**
CAS Name:	**N/A**
CAS Number:	**352010-65-5**



IUPAC Name:	**2-(2-Methoxy-ethoxymethyl)-6-trifluoromethyl-nicotinic acid**
CAS Name:	**N/A**
CAS Number:	**380355-55-5**